Standard Operating Procedure for the Determination of Ammonia

1.0 Scope and Applicability

This analysis is applicable to surface, drinking, saline water and domestic and industrial waste. This method is primarily intended for waste water. The curve is linear. The applicable range is 0.1 to 2 mg NH_4^+ /L. The Method Detection Level is 0.06 mg NH_4^+/L. The MDL was determined using seven replications of a matrix blank spiked with 0.25 mg NH_4^+/L. Each of the seven samples were distilled prior to running on the FIA.

2.0 Summary of Method

The pH of 10 mL of sample is adjusted to 9.5 and then the sample is buffered at a pH of 9.5 followed by distillation into a boric acid solution. The distillate is brought to a volume of 50 mL. The sample is then ready for analysis on the FIA.

On the FIA ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenolblue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration.

3.0 Definitions

Calibration blank - A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

Flow Injection Analysis (FIA) - references the Lachat equipment listed in (sec 6.1.1).

Laboratory Fortified Blank (LFB) - An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

Laboratory Reagent Blank (LRB) - Reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

Material Safety Data Sheet (MSDS) - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

Quality Control Sample (QCS) - A solution containing a known concentration of the analyte, usually from a source outside the laboratory and independent of the standards. The purpose of this solution is to verify that the standards are correct.

Reagent water - Water which has been run through a reverse osmosis system and then through de-ionization cartridges so that the final conductivity of the water is 17 meg ohm.

Stock Standard Solution - A concentrated solution containing the method analyte prepared in the laboratory using a purchased standard reference material, ideally traceable to NIST.

4.0 Interferences

All wastewater discharge samples must be distilled prior to color development. Calcium and magnesium ions may be present in concentrations sufficient to cause precipitation problems during analysis. The EDTA solution used in the buffer should prevent the precipitation. Samples that are turbid or colored may interfere. Turbid samples can be filtered and colored samples can be distilled.

5.0 Safety

WARNING: The handling of phenol in this method is subject to extra care as it can cause servere burns on the skin. It can also be absorbed directly through the skin. Exposure of large surface areas of the skin have been linked to death. Brain damage and other neurological problems have also been attributed to inhalation of vapors. Cardiac arrest is also a possibility. Use protective gloves and work under the hood while preparing this reagent. When running this chemistry on the FIA make sure the vent hood over the waste sink is operating and water is running down the sink. Normal laboratory precautions should be observed in handling other reagents in this method.

This method does not address all safety issues associated with its use. Laboratory management and staff are responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals

specified in this method. A reference file of material safety data sheets (MSDS's) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the analyst.

6.1 Instrumentation

6.1.1 Lachat QuikChem 8000 Automatic Flow Injection Analyzer
6.1.2 96 position random access sampler
6.1.3 Ismatec 16 position proportioning pump
6.1.4 ammonia injection module (see section 17 for diagram)
6.1.5 colorimeter with
10 mm flow cell
630 interference filter
190 cm sample loop of 0.022 inch ID PTFE
6.1.6 Gateway 2000 P5-60 computer
6.1.7 NEC MultiSync 3FGe monitor
6.1.8 HP 3Si printer
6.1.9 heater set to 60 deg C
6.1.10 omnion software (ver 2.0 Jan 1999)
6.1.11 Labconco RapidStill I Unit (manufacture # 65000-00)

6.2 Supplies

6.2.1 short range pH paper: Hydrion pH 9-10, (cat. No. 366), Micro Essential Laboratory, Inc.

7.0 Reagents and Standards

7.1 Distillation Reagents

7.1.1 Reagent Water:
Reagent water is obtained from one of the laboratory deionization systems.

7.1.2 Sodium Hydroxide 10N:
Dissolve 400 g of NaOH in a large beaker with 500 ml reagent water.

Cool with an ice bath. Bring to a final volume of 1 L with reagent water.

7.1.3 Borate Buffer:
Dissolve 9.5 g of sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) in 500 ml of water. Add 8.8 ml of 1N NaOH and dilute to 1L with reagent water..

7.1.4 Boric Acid:
Dissolve 20 g of boric acid (H_3BO_3) in a 1 L beaker with enough reagent water to make 1 L of solution.

7.2 FIA Reagents

7.2.1 Sodium Phenolate:
CAUTION: wear gloves when handling phenol as you can burn your skin. In a 2 L beaker add 176 ml of liquefied phenol to 1200 ml of reagent water. While stirring, slowly add 64 g of NaOH. Cool and dilute to 2 L. Degas with Helium.

7.2.2 Sodium Hypochlorite:
In a 1 L beaker containing 400 ml of reagent water add 500 ml of regular Clorox bleach (5.25%). Add reagent water to make 1 L of solution. Degas with He.

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7.2.3 Sodium Nitroprusside:
In a 2 L beaker add 4 g of sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot 2H_2O$) to 2 L of reagent water. Mix to dissolve. Degas with Helium.

7.2.4 Buffer:
In a 2 L beaker add 100 g EDTA (Ethylenediaminetetraacetic acid, disodium salt dihydrate):
$NaO_2CCH_2N(CH_2CO_2H)CH_2CH_2N(CH_2CO_2Na)CH_2CO_2H \cdot 2H_2O$ and 19.8 g sodium hydroxide (NaOH). Mix to dissolve. Degas with Helium.

7.3 Quality Control Reagents

7.3.1 Stock NH_3 Standard (1000 ppm N)
In a 1 L volumetric flask dissolve 3.8188 g of NH_4Cl that has been dried for two hours at 105 deg C in about 800 ml of reagent water. Bring to volume.

7.3.2 Spike solution: (100 ppm N)
Place 0.3819 g of NH_4Cl in a 1 L volumetric flask with about 800 ml of

reagent water. Dissolve then bring to volume. Add 0.25 ml of this spike solution to the wastewater discharge sample aliquot prior to changing the pH with NaOH. This will give you a 0.5 ppm ammonia spike.

7.3.3 Working Standards

Using the 1000 ppm stock standard (7.3.1) prepare a 100 ppm NH_3 standard monthly. Use this 100 ppm standard to prepare the following daily working standards in 100 ml volumetric flasks.

ml of 100 ppm	ppm NH_3
2	2
1	1
0.5	0.5
0.1	0.1
0	0

8.0 Sample Collection, Preservation and Storage

Samples should be preserved with H_2SO_4 to a pH <2 and stored at 4 ^{o} C. Maximum Holding time is 28 days.

9.0 Quality Control

The initial distillation for the day must be a reagent water blank. The purpose of this is to verify that there is no carry over of ammonia from the distillation glassware. The check sample should be distilled as well.

The correlation coefficient for the linear standardization must be 0.995 or higher. Improvements may be made to the regression by omitting an outlier. If this fails to improve the regression then new standards should be prepared. Each set of 10 samples will include 1 check sample, a duplicate and 1 spiked sample. The check sample needs to be distilled only once. The spiked sample is prepared by placing 0.25 ml of a 100 ppm spike solution (Sect. 7.3.2) into the receiving funnel of the distillation unit which previously had 10 ml of sample added. Duplicate, spike and check samples must be within acceptable limits. Check the QC charts generated by the LIMS for the 3 standard deviation window. Each run will include a laboratory fortified blank which must run within $\pm$ 10% of its true value. Percent recovery for spiked samples will be calculated by the LIMS computer as:

$R = [(C_s - C)/S] \times 100$

Where R = percent recovery

C_s = fortified sample concentration

C = sample background concentration
S = concentration equivalent of analyte added to the sample

This method has a tendency for the baseline to rise. This is probably due to the preservation acid in samples of surface waters which are not distilled prior to running on the FIA. To compensate for this the strength of the buffer (7.2.4) has been increased from 5.5 g NaOH/L to 9.9 g NaOH/L.

After the samples have been run on the FIA, review the results for the reagent water blank. If it seems unacceptably high redistill the two samples which followed it and compare the results before reporting any numbers. If they don't agree, re-distill the whole batch of samples.

10.0 Calibration and Standardization

10.1 Procedure

10.1.1 Prepare 5 standards including a calibration blank in reagent water as outlined in section 7.3.3.

10.1.2 Analyze by the procedure outlined in section 11.2.

10.2 Evaluation

The curve is linear. The correlation coefficient for the regression must be 0.995 or better. A single outlying standard may be omitted to make improvement in the curve. If this fails to correct the curve, additional steps should be undertaken including preparing new standards before proceeding. A check standard will be run after every 15 samples. If the check standard is within 5% of true value, the analysis can proceed. If it is within 8%, calibration will be redone before proceeding. If the check standard is outside 8% of error, all the standards will be rerun and all samples analyzed since the last acceptable calibration will be rerun. The preceding quality control (section 9.0) is run automatically under the omnion software to check the drift of the standards.

11.0 Procedure

11.1 Distillation:

11.1.1 Turn on the distillation unit and the cold water condenser. Check and refill the reagent water reservoir if needed. Steam out the unit for 10 min. Obtain fresh reagent water for all bottles.

11.1.2 Place 5 ml of the boric acid solution (7.1.4) and 20 ml of reagent water in a 50 ml disposable beaker.

11.1.3 Using a volumetric pipet, place 10 ml of sample into the top of the distillation unit. Add 5 drops of 10 N NaOH to the sample and check the pH with short range pH paper. When the pH is 9.5 or above open the waste valve and drain the main chamber to the drain.

11.1.4 Place the 50 ml receiving beaker under the distillation spout. Close the waste valve and open the sample valve. Rinse the sample into the boiling chamber with a small about of reagent water and quickly close the valve.

11.1.5 Raise the receiving beaker so that the spout is under the surface of the liquid. Place 5 ml of the borate buffer (7.1.3) into the sample holding cup. Slowly release this solution into the reaction flask watching so that the liquid in the condenser spout does not suck back up into the reaction flask. Trap a small amount of the buffer with the stopcock.

11.1.6 Set the clock and distill the sample for 4.0 min. During the last 30 seconds lower the beaker and allow the condensate to drip into the beaker. Rinse the spout.

11.1.7 Bring the sample distillate volume to 50 ml with a graduated cylinder and reagent water. Place the distilled sample on the bench and cover with a second empty beaker.

11.1.8 Rinse out the sample chamber with reagent water and place a waste beaker under the condensate spout. Steam out the distillation chamber two or three times before the next sample. Continue until all samples have been distilled.

11.2 FIA Procedure

11.2.1 Turn on the computer and all other components. Set the heater temperature to 60 deg C.

11.2.2 Install the ammonia manifold board, interference filter and sample loop. Connect all pump tubes. Pump reagent water and check for leaks

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11.1.3 Place standards into the autosampler in descending order. Load all samples.

11.2.4 Open the proper method (ammonia.met) and Tray Table (ammonia.tra) on the FIA computer.

11.2.5 Place all feed lines into the reagents and pump until a stable base line is obtained.

11.2.6 Transfer the work list to the FIA computer, by exporting the work list from a LIMS connected computer to a 3 ½ disk.

11.2.7 Import the work list from the 3 ½ inch disk to the FIA computer using the Start menu and selecting: "receive log numbers". Select "2. Final Export to Instrument". At the prompt provide a file name such as the current date: 0011070A. At the second prompt indicate that your first sample will start in position 6 following the standardization.

11.2.8 Exchange the current work list in the FIA computer memory with the new one imported in 11.2.7 by selecting File, "import Tray" and then selecting the file name used in 11.2.7 and selecting OK.

11.2.9 Check the tray table (work list) on the FIA computer for completeness of the transfer, dilutions that were made and deleting any unwanted items left over from the previous tray. All samples distilled will have a dilution factor of 5. Replace the old tray by saving the newly transferred one: "File", "Save Tray".

11.2.10 Start the analysis by clicking the "Run Tray" button on the main tool bar. On the next window click catalog on the file name line. On the next window type in your file name you used in 11.2.7. Click OK.

11.2.11 Calibration should start automatically. After the calibration has completed verify that the calibration curve is acceptable.

11.2.12 Check to see that the LFB is running within 10% of true value and that the QCS is running within the statistical window.

11.2.13 When the analysis is complete remove all pump lines from the reagents and place in reagent water for 5 or 10 min. Remove lines from the water and pump dry. Release pump tubes from the pump. If needed lines may be cleaned by pumping 1N HCl through them for 5 min followed by reagent water for 5 minutes. Contrad 70 Detergent (Fisher Scientific 04-355) is also effective in cleaning the lines.

11.2.14 Turn off the pump and all modules. Release the pump tube cassettes.

11.2.15 Print the calibration curve. From the tool bar click the graphic of the Calibration curve "Review". Select: "Print", "Current Analyte".

11.2.16 Print the custom report. From the tool bar click "Custom", "Print", "OK"

11.2.17 Export the data file. Select: "File", "Export Data". Select "channel 1 to 1" for export. Verify the file name used in 11.2.7 with the extension of .txt. Click ok.

11.2.18 Transfer the exported file and data back to a 3 ½ inch disk. From the Start Menu, select "Send Data". Select, "transfer data to floppy". Select "1 - import single channel data". Type in the data file name you used in 11.2.7.

11.2.19 Import the FIA data from the 3 ½ inch disk to the LIMS.

11.2.20 Instrument set up/method parameters

Chemistry	Direct
Injection to peak start	40
Peak base width	40
% width tolerance	20
Threshold	2000
Method cycle period	60
Probe in sample	20
Load period	13

12.0 Data Analysis and Calculations

All calculations for this procedure are performed by the FIA computer and software. Dilutions are entered into the omnion software on the FIA. Report all result as mg N/ L to three decimal places. All results including duplicates, spikes, quality control samples, LFB and check standards will be imported to the LIMS computer for report writing and later used in preparation of control charts. The distribution sheet from the LIMS will be kept as well as all original FIA data in the ammonia book by date of analysis.

13.0 Method Performance

The method detection level was calculated using 7 replications of a 0.25 mg NH_4^+ /L

matrix spike. The spike solution was treated as a sample and distilled prior to running on the FIA. The MDL was 0.06 mg NH_4^+ /L with a single operator precision of 0.0187 mg NH_4^+ /L. The bias at the spiked level was -37%.

14.0 Pollution Prevention

Phenol has a serious potential for pollution and only minimum quantities of sodium phenate should be prepared, as needed. Do no make more than can be used in 3 to 4 weeks.

15.0 Waste Management

It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW., Washington DC, 20036.

16.0 References

EPA (Aug 93) Method 350.1 (Colorimetric, Automated Phenate)
Lachat QuikChem Method No. 10-107-06-1-B (Dec 1993)

17.0 Tables, Diagrams, flowcharts, and Validation Data

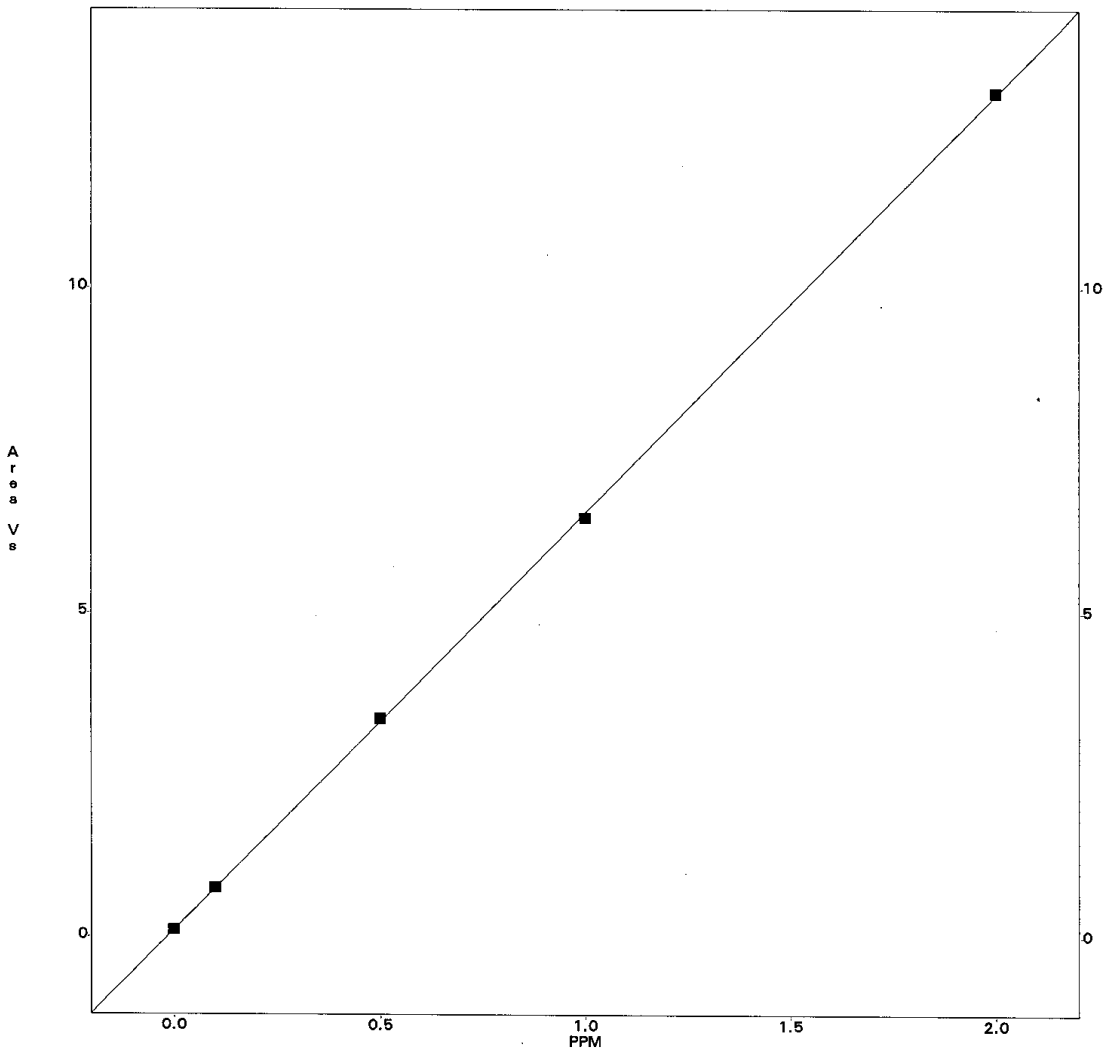
Raw data to be saved includes the printout of the mg/L for the samples from the FIA along with the regression analysis for the standards. After the data has been entered into the LIMS, the distribution sheet will also be kept.

Ammonia calibration curve

Lvl	Area	PPM	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replic STD	Replic % RSD	Residual 1st Poly
1	13008402	2.0	13008402					0.0	0.0	-0.2
2	6465250	1.0	6465250					0.0	0.0	1.3
3	3381114	0.5	3381114					0.0	0.0	-1.5
4	763515	0.1	763515					0.0	0.0	-0.8
5	114803	0.0	114803					0.0	0.0	

1st Order Poly
Conc = 1.555e-007 Area - 1.788e-002
r = 1.0000

Scaling: None - Weighting: None



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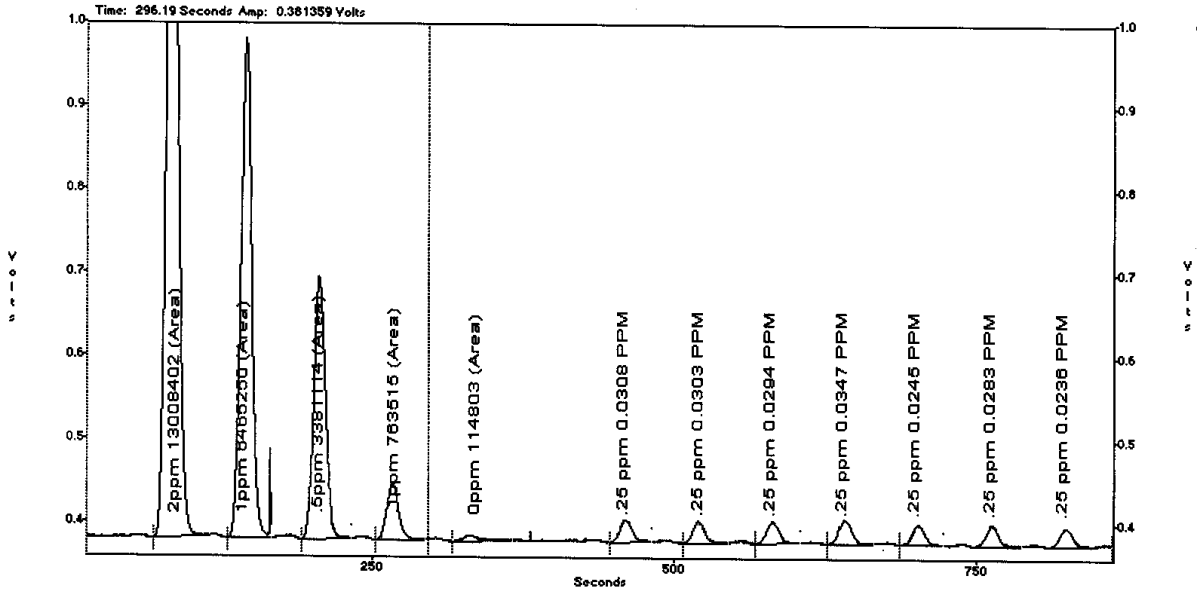
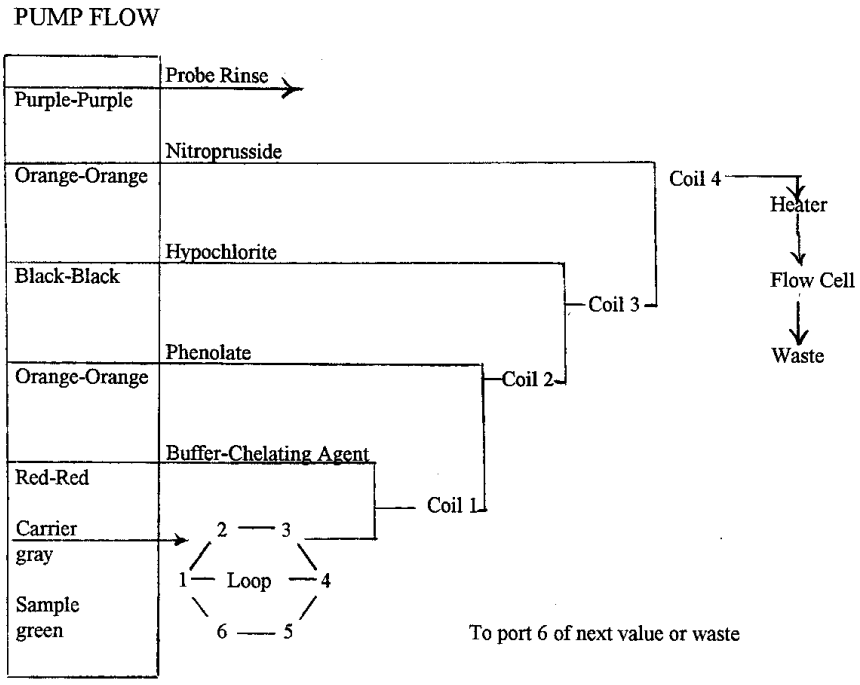
MDL for Ammonia



n = 7
mean = 0.1573 mg NH_4^+ /L
σ = 0.01869 mg NH_4^+ /L
bias at 0.25 mg NH_4^+ /L = -37 %
MDL = (0.01869)(3.14) = 0.0587 mg NH_4^+ /L

Ammonia Manifold Diagram



Notes

Sample loop = 190 cm of 0.022 inch inside dia PTFE tubing
Interference filter = 630 nm
Coils 1 and 3 contain 270 cm of 0.032 inch i.d. PTFE tubing
Coils 2 and 4 contain 180 cm of 0.032 inch i.d. PTFE tubing
Pump speed =35
Heater set at 60 deg C with 650 cm of 0.032 inch ID tubing.